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I. Hydrolysis test

Purpose: Test the chemical stability of both native and linear types of Hv1a and Hv1a-lectin in neutral PBS solvent (phosphate buffered saline, pH=7.4) in 4℃ for 1 day and 7 days

Materials: purified protein solutions of Hv1a and Hv1a-lectin, 1X PBS (pH=7.4)

Preparation:

A. Native protein solution
    1. Dissolve the purified protein solution in 1X PBS (pH=7.4)
    2. Stored in 4℃ refrigerator
B. Linear protein solution
    1. Dissolve the purified protein solution in 1X PBS (pH=7.4)
    2. Add βME (1:50) and boil up for 15 min
    3. Stored in 4℃ refrigerator

Note: Linear form protein will degrades in 4℃, so the experiment must be done right after preparation.

Procedures:

1. Store the solutions into 1.5 mL eppendorfs
2. Before store in 4℃ refrigerator for 0 (negative control), 1, 7 days, add pure glycerol (1:1) into each eppendorf and then store in -80℃ refrigerator
3. Run SDS-PAGE of totally 6 samples and 250 uM BSA (Bovine serum albumin) as standard sample
4. Scan the gel
5. Use ImageJ to calculate relative concentration

II. Proteolysis test－Enzymatic Stability

Purpose: Test the enzymatic stability of both native and linear types of Hv1a and Hv1a-lectin by applying Trypsin

Materials: purified protein solutions of Hv1a and Hv1a-lectin, 1X PBS (pH=7.4), 0.25% Trypsin-EDTA

Preparation: Native protein solution, Linear protein solution (As mentioned preciously)

Procedures:

1. Store the solutions into 1.5 mL eppendorfs
2. Add 0.25% Trypsin-EDTA (1:250) into tested eppendorfs
3. Incubate the solution in 37℃ incubator for 0 (negative control) and 1 day, and then incubate in 95℃ for 10 min to inactive protease
4. Store the samples in 4℃ refrigerator
5. Run SDS-PAGE of totally 6 samples and 250 uM BSA (Bovine serum albumin) as standard sample
6. Scan the gel
7. Use ImageJ to calculate relative concentration

III. Proteolysis test－Degradation rate

Purpose: Test the degradation rate of both native and linear types of Hv1a and Hv1a-lectin by applying Trypsin in the period of 4 hours

Materials: purified protein solutions of Hv1a and Hv1a-lectin, 1X PBS (pH=7.4), 0.25% Trypsin-EDTA

Preparation: Native protein solution, Linear protein solution (As mentioned preciously)

Procedures:

1. Store the solutions into 1.5 mL eppendorfs
2. Add 0.25% Trypsin-EDTA (1:250) into tested eppendorfs
3. Incubate the solution in 37℃ incubator for 0 (negative control) 0.5, 1, 2, 4 hours, and then incubate in 95℃ for 10 min to inactive protease
4. Store the samples in 4℃ refrigerator
5. Run SDS-PAGE of totally 6 samples and 250 uM BSA (Bovine serum albumin) as standard sample
6. Scan the gel
7. Use ImageJ to calculate relative concentration

IV. UV radiolysis test

Purpose: Test the degradation rate of both native proteins by applying UV light in the period of 2 hours

Materials: purified protein solutions of 6 proteins, 1X PBS (pH=7.4)

Preparation: Native protein solution, Linear protein solution (As mentioned preciously)

Procedures:

1. Store the solutions into 1.5 mL eppendorfs
2. Apply the tested samples to transilluminator (302 nm, 50 mW/m^2) for 0, 0.5, 1, 2, 4 hours, and then put in room temperature
3. Run SDS-PAGE of totally 6 samples and 250 uM BSA (Bovine serum albumin) as standard sample
4. Scan the gel
5. Use ImageJ to calculate relative concentration

V. Nature test

Purpose: Test the degradation level of both native and linear types of Hv1a and Hv1a-lectin under sunlight for 4 hours

Materials: purified protein solutions of Hv1a and Hv1a-lectin, 1X PBS (pH=7.4)

Preparation: Native protein solution, Linear protein solution (As mentioned preciously)

Procedures:

1. Store the solutions into 1.5 mL eppendorfs
2. Put the samples on a wide square in a transparent and closed acrylic box for 4 hours at different time
3. Run SDS-PAGE of totally 7 samples and 250 uM BSA (Bovine serum albumin) as standard sample
4. Scan the gel
5. Use ImageJ to calculate relative concentration